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(54) Title: ANALOGS OF VITAMIN E

(57) Abstract

The present invention relates to analogs of vitamin E having antiproliferative activity using human breast cancer cell line, MCF7. Compared to vitamin E, the new analogs of the present invention have a potent antiproliferative activity against human breast cancer cells.

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ANALOGS OF VITAMIN E

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to analogs of vitamin E and their use as pharmacological agents.

(b) Description of Prior Art

It is well known that reactive oxygen derived free radicals (i.e. $^{\bullet}OH$, $^{\bullet}OOH$, $^{\bullet}O_2^-$) are responsible for damaging cellular components and play an important role in initiating biological disorders leading to cancer, cardiovascular disease and aging (Halliwell, B; Gutteridge, J. M. C., Eds. In Free Radicals in Biology and Medicine, Clarendon Press: Oxford, 1989; Ames, B. N et al., *Proc. Natl. Acad. Sci.* **1993**, *90*, 7915). Vitamin E (α-Tocopherol, or α-TOH, Formula I-1) is one of the major fat-soluble antioxidants found in mammalian cells, and it plays a vital role in the maintenance of cellular redox status (Burton, G. W. et al., *Acc. Chem. Res.* **1986**, *19*, 194). Its stability is enhanced by protecting the phenolic hydroxyl group as an acetate derivative (Formula I-2) which upon *in vitro*, or *in vivo* enzymatic hydrolysis (for example, *cholesterol esterase*) releases the free phenol (Moore, A. N. J. et al., *J. Am. Chem. Soc.* **1995**, *117*, 5677; Moore, A. N. J. et al., *J. Am. Chem. Soc.* **1994**, *116*, 6945; Kahalka, H. A. et al., *J. Am. Chem. Soc.* **1991**, *113*, 2797). Increasingly, attention is turning to the role that this natural antioxidant, and its analogs, may play in reducing the incidence of heart disease and cancer (Bolkenius, F. N. et al., *Free. Rad. Res. Comms.* **1991**, *14*, 363; Grisar, J. M. et al., *J. Med. Chem.* **1991**, *34*, 257; Grisar, M .J. Bolkenius, F. EP 0 535 283 A1, **1991**; Grisar, M .J. Bolkenius, F. *EP 0 550 292 A1*, **1992**; Sen, C.K. et al. *FASEB J.* **1996**, *10*, 709; Irani, K. et al., *Science*, **1996**, *275*, 1649).

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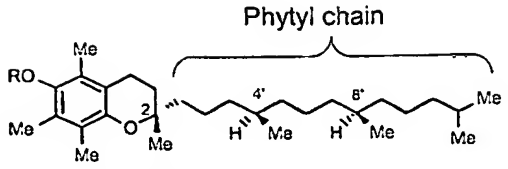


R: H, Vitamin E (RRR- α-Tocopherol, α-TOH, 1)
R: Ac, (RRR- α-Tocopheryl acetate, α-TOAc, 2)

A major limitation of the use of Vitamin E is its extreme insolubility in water. This limitation severely affects its pharmacokinetics and tissue pharmaco-distribution.

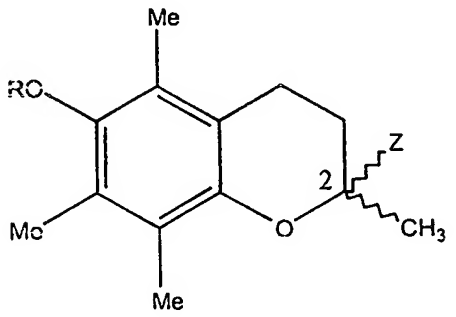
SUMMARY OF THE INVENTION

One aim of the present invention is to provide analogs of vitamin E which have improved characteristics while retaining the desirable features of Vitamin E.

In particular the analogs may exhibit characteristics such as efficient delivery or cell-uptake.

The invention also provides novel pharmaceutical formulations containing the analogs as active ingredient and processes for producing the analogs.

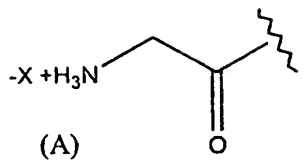
In accordance with the invention there is provided a compound of formula (X).



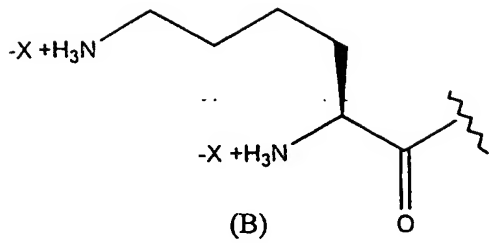
(X)

the 2-position is S or R racemic,

wherein R is



(A)



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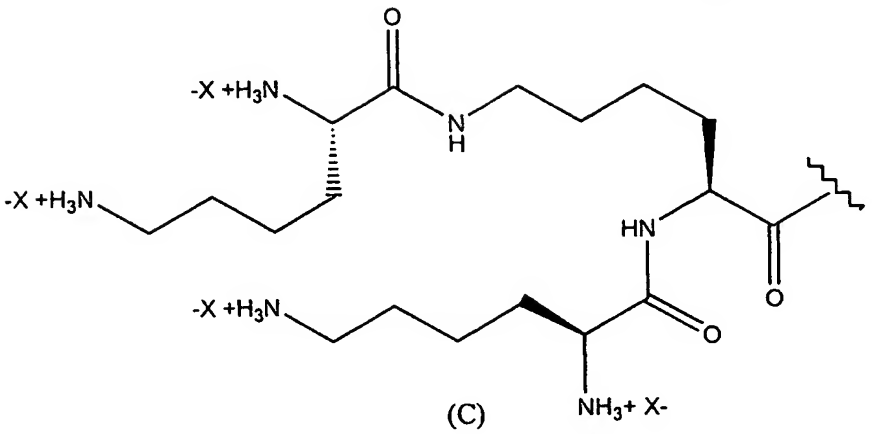

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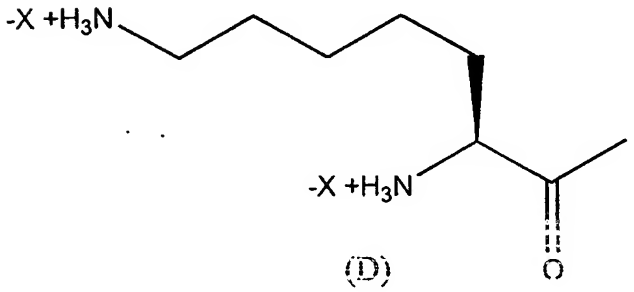


(D)

X^- is a pharmacologically acceptable anion, for example, chloride, bromide, brosylate, mesylate or tosylate;

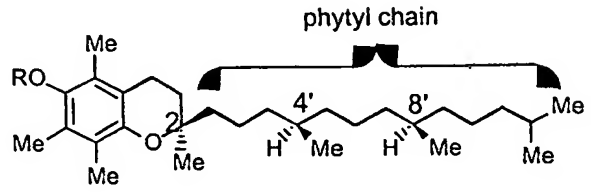
Z is CH_2OR_1 in which

R_1 is H, lower alkyl of 1 to 6 carbon atoms, lower acyl in which the alkyl moiety has 1 to 6 carbon atoms, or OR_1 is cholate ($C_{24}H_{39}O_5$);

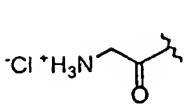
or

Z is 4,8,12 trimethyltridecyl (TMT) or a natural phytyl group.

The preferred analogs (3) to (9) of the present invention are as follows:



R:



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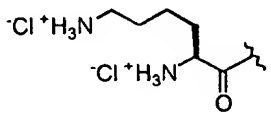

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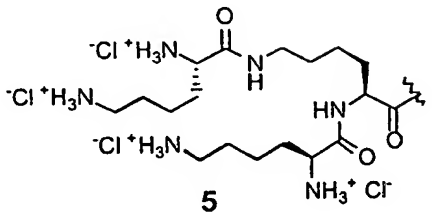

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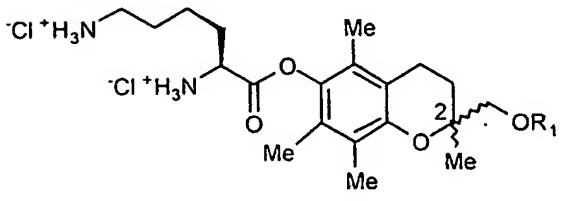
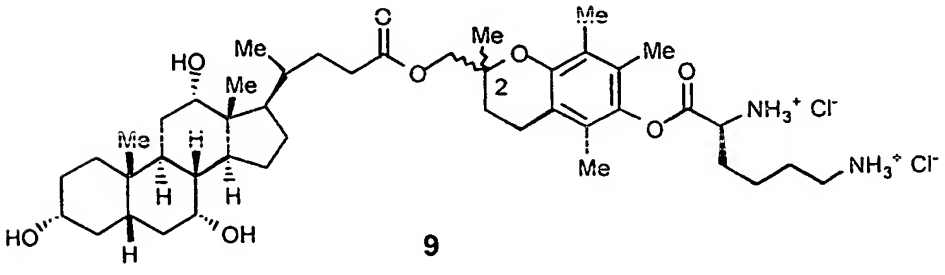


	R_1:
6	H
7	nC_5H_{11}
8	C(O)-nC_4H_9



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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a representation of vitamin E and its acetate showing the relationship with analogs (3), (4) and (5) of the invention;

Fig. 2 shows the nucleus of vitamin E and the regions which have been varied in accordance with the invention, together with representations of analogs (6), (7), (8) and (9) of the invention;

Fig. 3 shows activity of analogs of the invention against human breast cancer cell line; and

Fig. 4 demonstrates the antimetastatic effect of compound 4.

DETAILED DESCRIPTION OF THE INVENTION

In the preparation of the analogs (X) of the invention attention was directed to the derivatization of the phenolic hydroxyl of vitamin E by an ester bond, and modulation of the length of the 4,8,12-trimethyltridecyl chain, and its attachment to the central core of vitamin E (Fig. 1).

The analogs of the present invention have been designed to display efficient delivery and cell-uptake behavior while retaining the desirable antioxidant features of vitamin E. Two important factors were considered for our synthetic strategy (Fig. 2) (i) the derivatization of the phenolic hydroxyl of vitamin E with amino acid derivatives via an ester bond, and (ii) modulation of the nature and length of the phytyl chain. Derivatization of the phenolic hydroxyl of vitamin E as an amino acid conjugate introduces a positively charged group which is expected to be cleaved upon *in vitro* or *in vivo* enzymatic hydrolysis (e.g. by cholesterol esterases). The modulation of the chain length may reduce the membrane-philicity of Vitamin E

and enhance the solubility of analogs of Vitamin E in an aqueous media.

Particularly preferred analogs (X) of the invention in which Z is 4,8,12-trimethyltridecyl and R_3 is methyl include:

Analog (3) in which R is (A) and X^- is Cl^-;

Analog (4) in which R is (B) and X^- is Cl^-; and

Analog (5) in which R is (C) and X^- is Cl^-.

Particularly preferred analogs (X) of the invention in which Z is OR^1 are those in which R_3 is methyl and R is (D), X^- being Cl^- and include:

Analog (6) in which R_1 is H;

Analog (7) in which R_1 is nC_5H_{11}; and

Analog (8) in which R_1 is $CO.nC_4H_9$.

Another preferred analog (X) of the invention is

Analog (9) in which R is (B) wherein X^- is Cl^-, R^3 is Me and OR_1 is cholate.

Analogs of Vitamin E (3-9) have been tested for their antiproliferative activity using a human breast cancer cell line, MCF7, and compared with the commercially available vitamin E derivatives, i.e. vitamin E (1), vitamin E-acetate (2), vitamin E-succinate (3) and *rac*-Trolox (13).

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

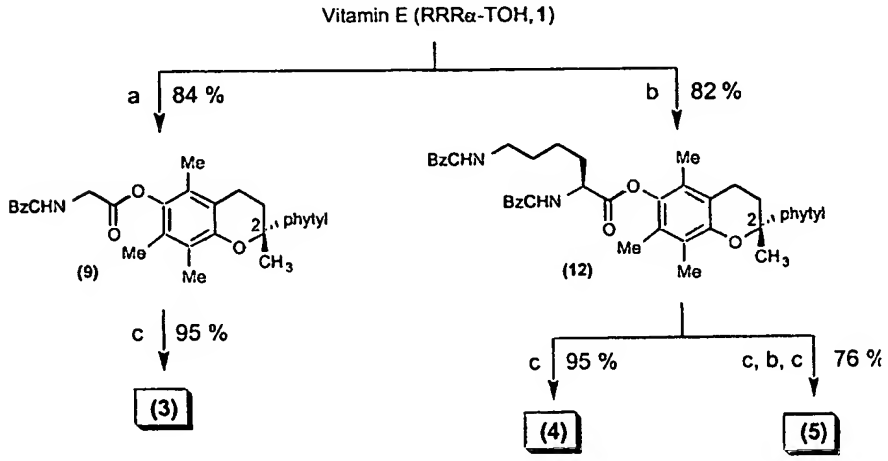
EXAMPLE I

Synthesis of Glycine or Lysine Conjugate of Vitamin E (Scheme 1, 3-5)

The CBz-glycine or di-CBz lysine ester of Vitamin E (17 and 12) was prepared in 82-84% isolated yield after purification over silica gel by coupling Vitamin E (1) to the CBz-glycine (10) or di-CBz-lysine (11)

using DCC/DMAP reaction conditions. All the new compounds were well characterized by ^{1}H NMR, ^{13}C NMR and MS analysis. 17 and 12 on hydrogenation conditions (H_2, 10% Pd/C in 95% EtOH) gave the corresponding free amine derivatives and were isolated as hydrochloride salts (3 and 4) after acidification with dil HCl. Compound 5 was obtained from 12 in 76% isolated yield in four steps: (i) hydrogenation of 12 to obtain the free amine derivative, (ii) coupling with the di-CBz-lysine (11) using DCC/DMAP reaction conditions, (iii) hydrogenation to obtain the free amine derivative, and (iv) the hydrochloride salt formation.

Scheme 1



Scheme 1: (a) CBz-Gly (10), DCC, DMAP (10 mol%), CH_2Cl_2; (b) di-CBz-Lys (11), DCC, DMAP (10 mol%), CH_2Cl_2; (c) (i) H_2, 10% Pd-C, 95% EtOH; (ii) dil HCl

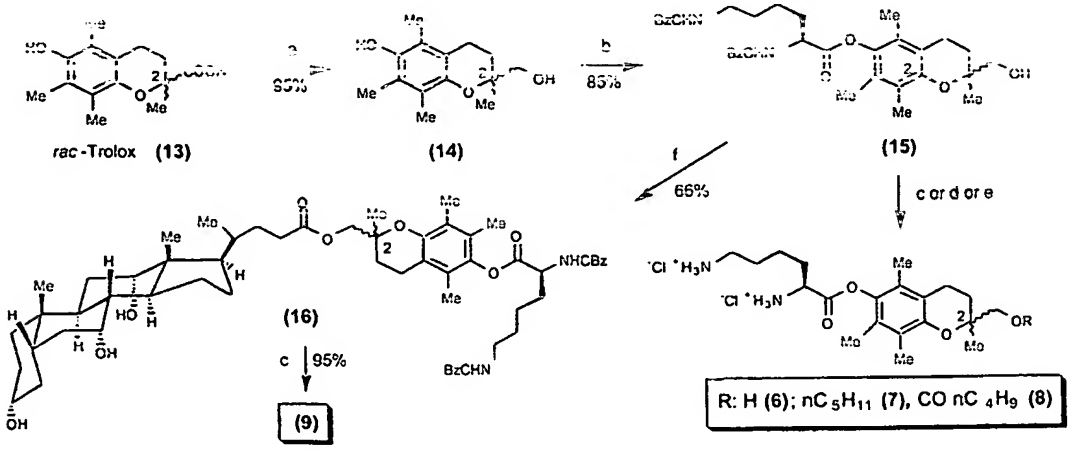
EXAMPLE II

Synthesis of Analogs of Vitamin E (Scheme 2, 6-9)

14 was prepared from the *rac*-Trolox (13) in 95% isolated yield in two steps, employing esterification followed by the LAH reduction. Di-CBz lysine ester derivative (15) was obtained from the coupling of

14 with the di-CBz-lysine (11) using DCC/DMAP reaction conditions in 85% isolated yield after purification over silica gel. All the new compounds were well characterized by ^{1}H NMR, ^{13}C NMR and MS analysis. 15 on hydrogenation conditions (H_2, 10% Pd/C in 95% EtOH) gave the corresponding free amine derivative, and was isolated as the hydrochloride salt (6) after acidification with dil HCl. The primary hydroxyl group of 15 was subjected to alkylation ($nC_5H_{11}Br$, Et_3N, RT) and acylation (nC_4H_9COCl, Et_3N, RT) reaction conditions separately. After purification over silica gel, both the products were subjected to the hydrogenation followed by the hydrochloride salt formation to obtain 7 and 8. 15 was also coupled with cholic acid using DIC, DMAP reaction conditions in order to introduce an amphiphilic auxiliary at the tail of Vitamin E derivative. 16 as a coupled product was obtained in 66% isolated yield after purification over silica gel by column chromatography. As in previous cases, the CBz-groups were removed by hydrogenation to obtain the free amine derivative which was isolated as the hydrochloride salt (9).

Scheme 2



Scheme 2: (a) (i) *p*TSA, EtOH, reflux; (ii) LAH, Et_2O; (b) di-CBz-Lys (**11**), DCC, DMAP (10 mol%), CH_2Cl_2; (c) (i) H_2, 10% Pd-C, 95% EtOH; (ii) dil HCl ® (**6**); (d) (i) $nC_5H_{11}Br$, Et_3N, THF; (ii) (c) © (**7**); (e) (i) nC_4H_9COCl, Et_3N, THF; (ii) (c) ® (**8**); (f) cholic acid, DIC, DMAP (10 mol%), THF (**9**).

EXAMPLE III

Antiproliferative Activity of Analogs of Vitamin E

Analogs of vitamin E (**3-9**) were tested for the antiproliferative activity on human breast cancer cell line MCF7 (Table 1). Cells were grown in RPMI medium, supplemented with 10% fetal bovine serum and antibiotics, and were cultured in 5% CO_2 (Alaoui-Jamali, M. A. et al., *Radiation Res.* **1992**, *129*, 37). For antiproliferative activity, MCF7 cells were seeded at the density of $1x10^3$/100ml/well in 96 well plates. After 18 h of culture, the cells were treated with various concentrations of vitamin E analogs, **3-9** for 96 h. The cytotoxicity was evaluated using 3(4-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Zheng-Rong, N et al., *Cancer Res.* **1995**, *55*, 4760). In brief, the culture media was replaced with a solution composed of 20 µl complete media and 20 µl of a solution containing 2.5 mg/ml of MTT in phosphate buffer (pH 7.4). After 4h of incubation at 37°C, 100 µl of DMSO was added to dissolve the precipitate of reduced MTT. The absorbance was determined at 570 nm with a micro plate reader (BIORAD-450). The IC_{50} was calculated as the dose of each analog causing a 50% reduction in absorbance, in comparison to untreated cells or cells treated with the solvent alone.

Table 1

Antiproliferative Effects of Vitamin E Derivatives and its Analogs

Compound	Human Breast Cancer Cell Line MCF 7 IC_{50} (μM)
Vit E-OH (1)	329±12
Vit E-OAc (2)	>500
Vit E-O-succinate	>368
Trolox (13)	1461±246
3	-
4	12±2
5	-
6	194±62
7 (water soluble)	22±6
8 (water soluble)	15±1
9 (water soluble)	4±1

In comparison to Vitamin E-OH (1), Vitamin E-OAc (2), Vitamin E -O-succinate (purchased from Sigma) and the *rac*-Trolox (13), the new analogs 4, 7 and 9 have shown a high activity against human breast cancer cell line (Table 1, Fig. 3). Simple replacement of the acetate (2) of Vitamin E by the lysine conjugate (4) resulted in a dramatic shift in the antiproliferative activity. Furthermore, replacement of the 4,8,12-trimethyltridecyl group of the lysine conjugate, 4, by a short hydrocarbon chain attached by an ether or an ester bond (7 or 8) or by an amphiphilic auxiliary (9) enhanced the solubility at physiological *p*H without affecting the antipro-liferative activity. As expected, *rac*-Trolox (13), in which the 4,8,12-trimethyltridecyl chain of Vitamin E has been replaced by the -COOH group, was not active. Similar results were

observed with the human breast cancer cell line, T47D and colon cancer cell line HCT116.

EXAMPLE IV

***In vivo* study using the Lewis lung carcinoma model**

Cell culture

The Lewis lung carcinoma clone, M47, with a high metastatic potential to the lung. These cells were confirmed to be free of mycoplasma infection. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, under 5% CO_2. Cells were passaged twice a week. Stocks of cells were generated and stored as early passages (passage no. 8-10). Cells were then propagated and stocks of the same passages were established and stored in liquid nitrogen for further studies.

For tumor induction, cells were grown to 70% confluence in complete medium and then collected using trypsin-EDTA solution [0.05% trypsin, 0.53 mM EDTA-4Na in HBSS without Ca++, Mg++, and $NaHCO_3$; Cellgro no. 25-052-Li]. Cells were then centrifuged and washed three times with phosphate buffer solution [D-PBS, Ca++ and Mg++ free; Cellgro no. 21-031-LV], and resuspended at a dilution of 0.1 to $1x10^6$ cells/0.1ml. Viability was examined by trypan blue staining and only flasks in which the viability was >95% were used for *in vivo* studies.

The mouse strain used in this study is C57BL/10 from the research laboratories and incinerators, and access to the animal facility is strictly limited to animal users only. Animal room used in our studies has two doors, one serving as the entrance, and the other door provides direct access to washing/sterilization/incineration facilities. It permits accurate adjustment of environmental parameters includ-

ing temperature, humidity, ventilation, and lighting. Cleaning and sanitation practices are performed, on a daily basis, by personnel with appropriate training.

Tumor cell inoculation and treatment

Animals were housed 5 per cage and were fed a diet of animal chow and water ad libitum. After one week acclimatization, LLC cells were transplanted subcutaneously, as a suspension of tumor cells [2-5x105 viable cells per 0.1ml], in the axillary region of the right flank. All animals were inoculated at the same site. Animals were subjected, on a daily basis, to general examination. Tumor growth was monitored every second or third day using calipers. Parameters measured are: tumor measured along the longest axis (length) and the perpendicular shortest axis (width) and the relative tumor volume (in cm^3) was calculated by the formula: [Length (cm) x (width cm)^2]/2. When the tumor reaches a size of 0.5-1.0 cm2 (approximately 2-3 weeks), mice were randomized into three groups.

Animals were subjected to surgery to remove the primary tumor. The mice were lightly anesthetized with Forane. The skin overlying the tumor was cleaned with betadine and ethanol, in a laminar flow hood. A small skin incision (0.5-1cm) was made using a sterile scalpel, and the tumor was carefully separated from the normal tissues (skin and muscle). LLC (at early stage of growth; 1-3 weeks) is well localized tumor and separation was easy to achieve without any significant damage to normal tissues. The tumor was removed, weighed and fixed for histopathology purposes. The wound was closed with surgical stainless steel clips (Autoclips; 9mm; Clay Adams, Inc., Parsippany, NJ). This site was further disinfected with betadine and the animal was housed as described earlier.

Mice were randomized after surgery into a group of 5 per cage. Cages were randomly assigned to specific experimental groups. The mice were then labeled by numbers using the "ear punching" method. Mice were checked on a daily basis to ensure the absence of infection. Animals with discomfort were sacrificed immediately. An additional extra-group of control mice was included to determine the optimal timing for sacrifice in order to obtain a significant number of well localized lung metastases. This group was subjected to the same experimental procedure as group 1 with the exception of drug treatment. Based on this group, a period of two weeks after removal of the primary tumor was sufficient to obtain an average of 20-30 nodules on the lung surface. Therefore, a two week period after primary tumor removal was used to sacrifice mice of group 1.

Dosing schedule and treatment

Drugs were given by intraperitoneal [total volume of 0.5ml per animal, every second day after surgery, for the duration of the experiment. Control animals were given the same volume of saline solution [0.9% sodium chloride; Abbott Lab., lot no. 12 455 WS]. The dose of each drug was normalized to an average of 20g body weight per animal.

Animal sacrifice, tumor/organs preparation: At the end of each experiment (a total of 5-6 weeks), animals were sacrificed in a CO-2 Chamber and autopsied. Tumors, organs or both were removed under sterile conditions [using a laminar flow hood]. Tumors were weighed. Organs (5 per group) were examined for gross pathological changes and then fixed in 10% formalin. Lungs were fixed in 10% Bouin's fixative diluted in a formalin solution, and lung surface metastases were counted using a stereomicroscope at 4x magnification or a magnifying-glass, and then lungs were embedded in

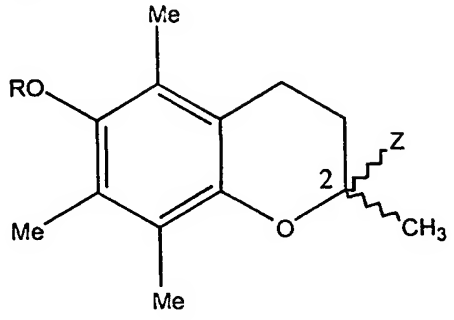
paraffin wax according to standard procedures. Embedded tissues were stored for future histopathological studies.

The results are illustrated in Fig. 4. The results demonstrate that treatment of mice with compound 4 at doses of 1 or 10 mg/Kg body weight, given as an intraperitoneal injection, resulted in an approximately 50% of lung metastases, compared to control mice treated with the solvent alone. No toxic effect was observed at these doses.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

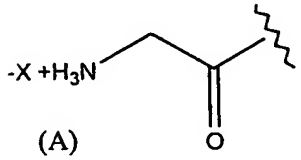
1. A compound of formula (X) provided a compound of formula (X):



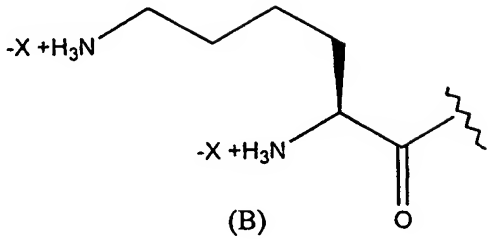
(X)

the 2-position is S or R racemic,

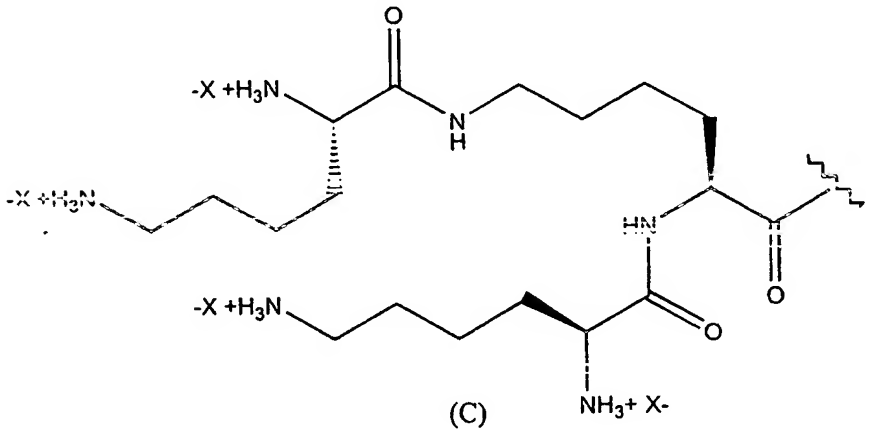
wherein R is



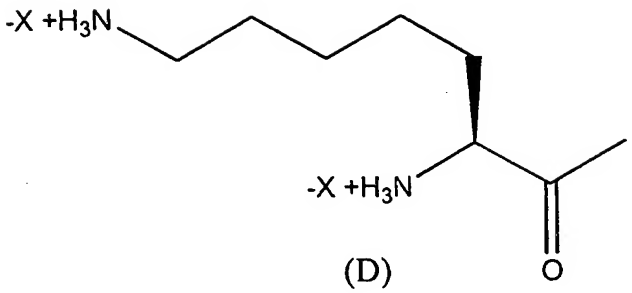
(A)



(B)



(C)



(D)

X^- is a pharmacologically acceptable anion, for example, chloride, bromide, brosylate, mesylate or tosylate;

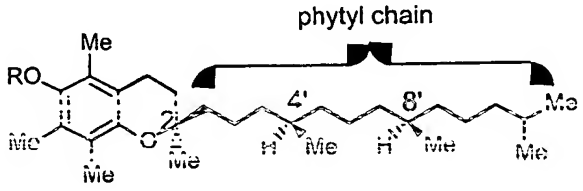
Z is CH_2OR_1 in which

R_1 is H, lower alkyl of 1 to 6 carbon atoms, lower acyl in which the alkyl moiety has 1 to 6 carbon atoms, or OR_1 is cholate ($C_{24}H_{39}O_5$);

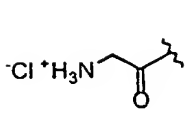
or

Z is 4,8,12 trimethyltridecyl (TMT) or a natural phytyl group.

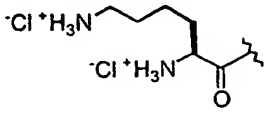
2. A compound of claim 1, wherein said compound of formula X and R are as follows:



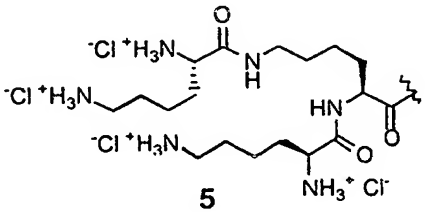
R:



3

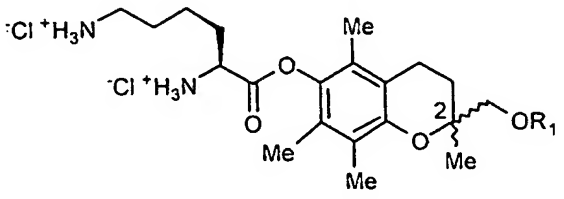


4

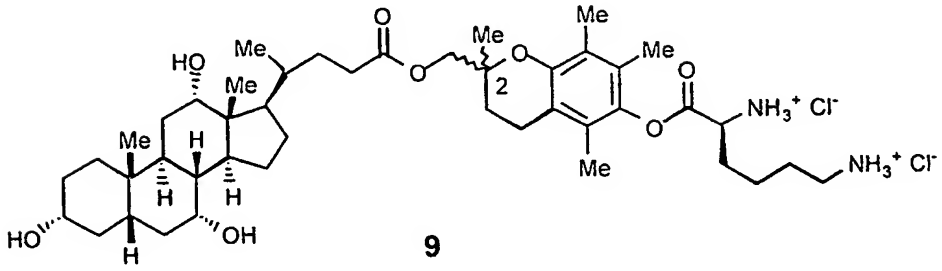


5

3. The compound of claim 1, wherein said compound of formula X and R are as follows:



	R_1:
6	H
7	nC_5H_{11}
8	C(O)-nC_4H_9



9

4. A pharmaceutical composition comprising a physio-logically acceptable, therapeutically effective amount of a compound of formula (X) of claim 1, in association with a pharmaceutically acceptable carrier.

5. A pharmaceutical composition comprising a physio-logically acceptable, therapeutically effective amount of any one of analogs (3) to (9) of claims 2 and 3, in association with a pharmaceutically acceptable carrier.

6. A compound according to claim 1, for use in the treatment of cancer.

7. Use of an analog (3) to (9) of claims 2 and 3, in the manufacture of a medicament for the treatment of cancer.

8. A method of treating cancer comprising administering to a person in need of treatment, a therapeutically effective amount of a compound of claims 1 to 3.

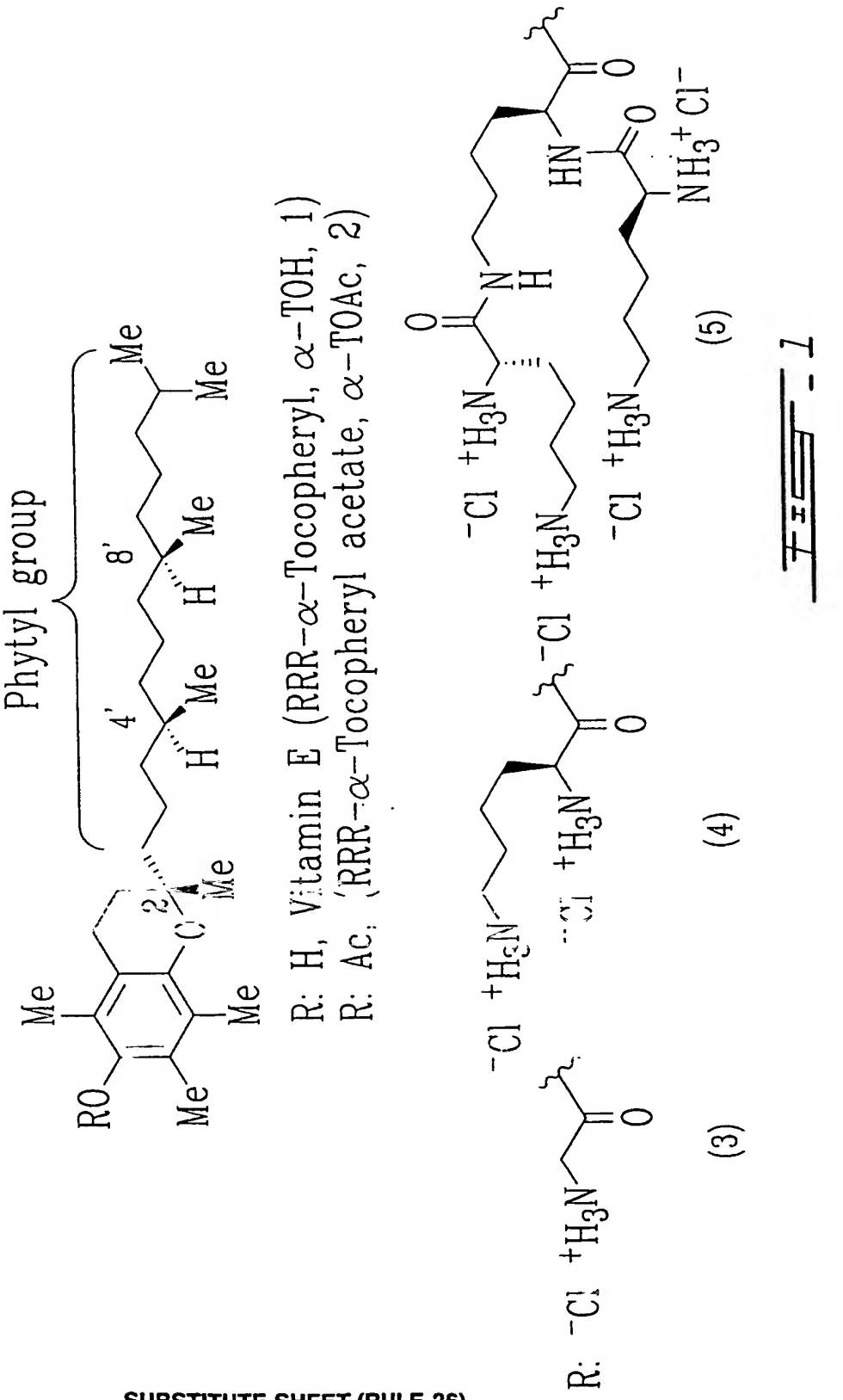
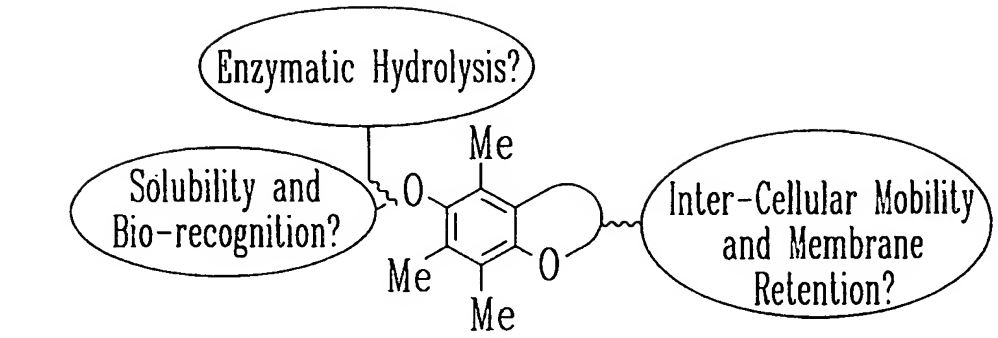
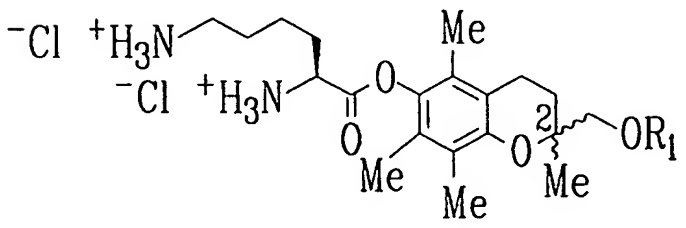
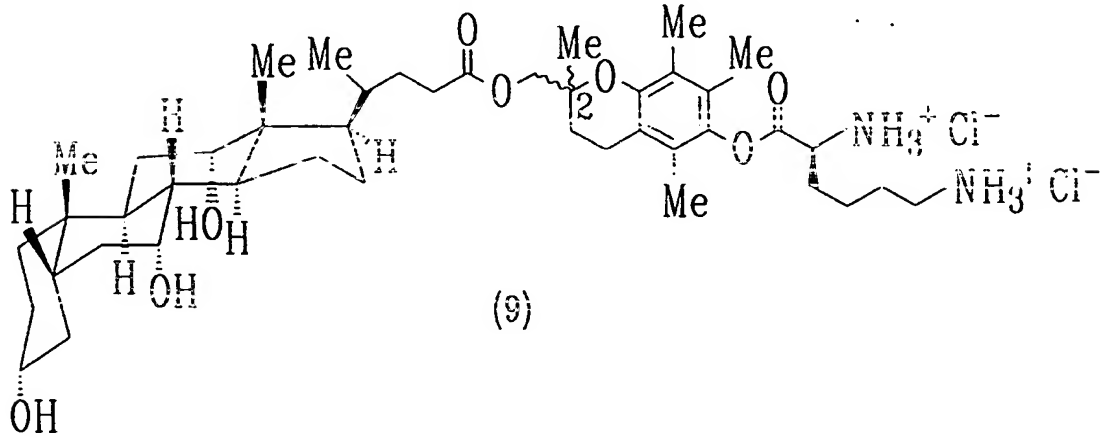

Phytyl group
RO
Me
Me
Me
Me
Me
Me
Me
Me
H
H
2'
4'
8'
R: H, Vitamin E (RRR-α-Tocopheryl, α-TOH, 1)
R: Ac, (RRR-α-Tocopheryl acetate, α-TOAc, 2)
R: -Cl +H3N
O
(3)
(4)
(5)
HN
NH3+ Cl-

Fig - 1





R_1:
H (6)
nC_5H_{11} (7)
CO nC_4H_9 (8)



(9)

Fig. 2

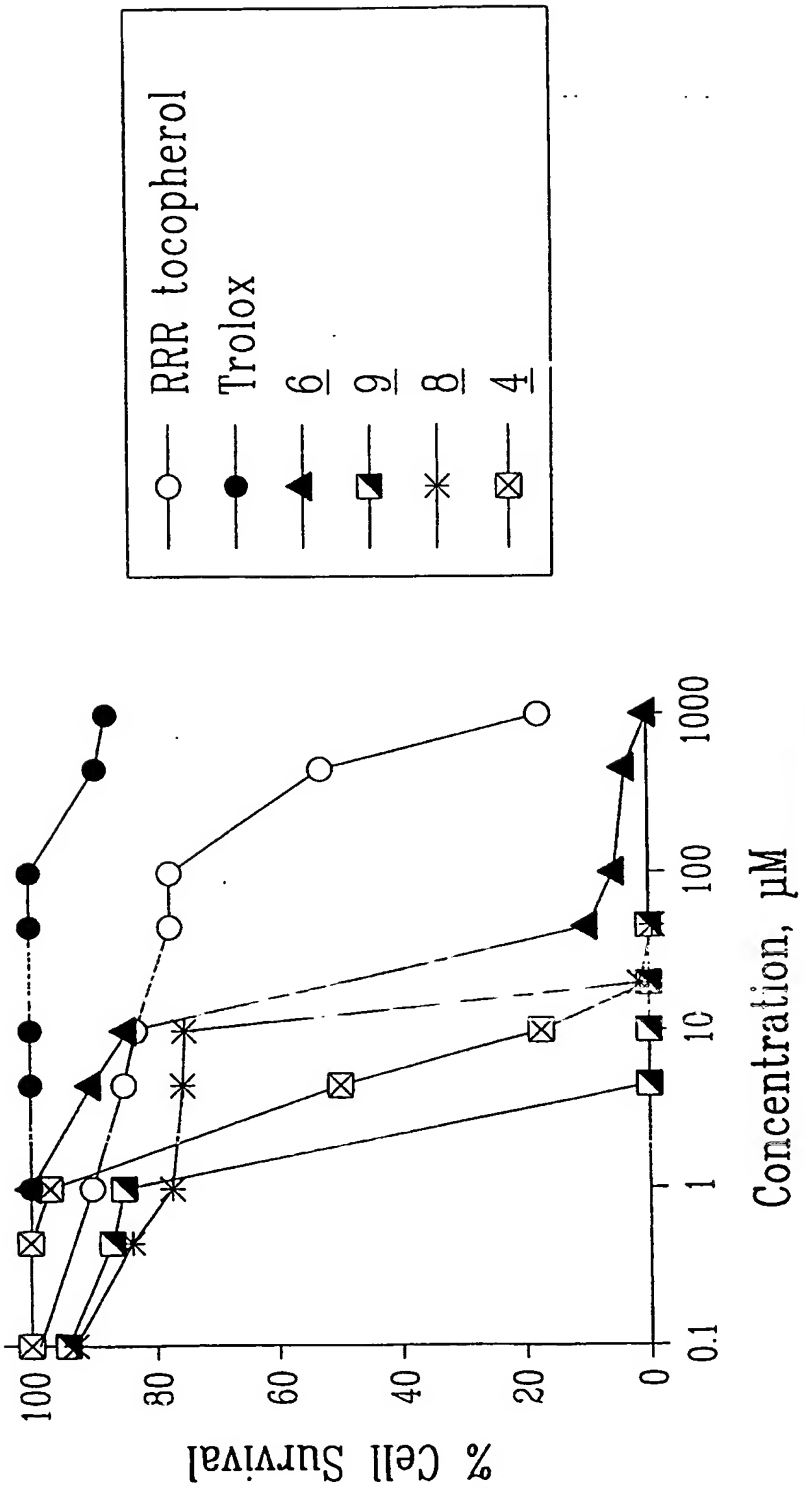

RRR tocopherol
Trolox
6
9
8
4
% Cell Survival
100
80
60
40
20
0
0.1
1
10
100
1000
Concentration, μM

Fig - 3

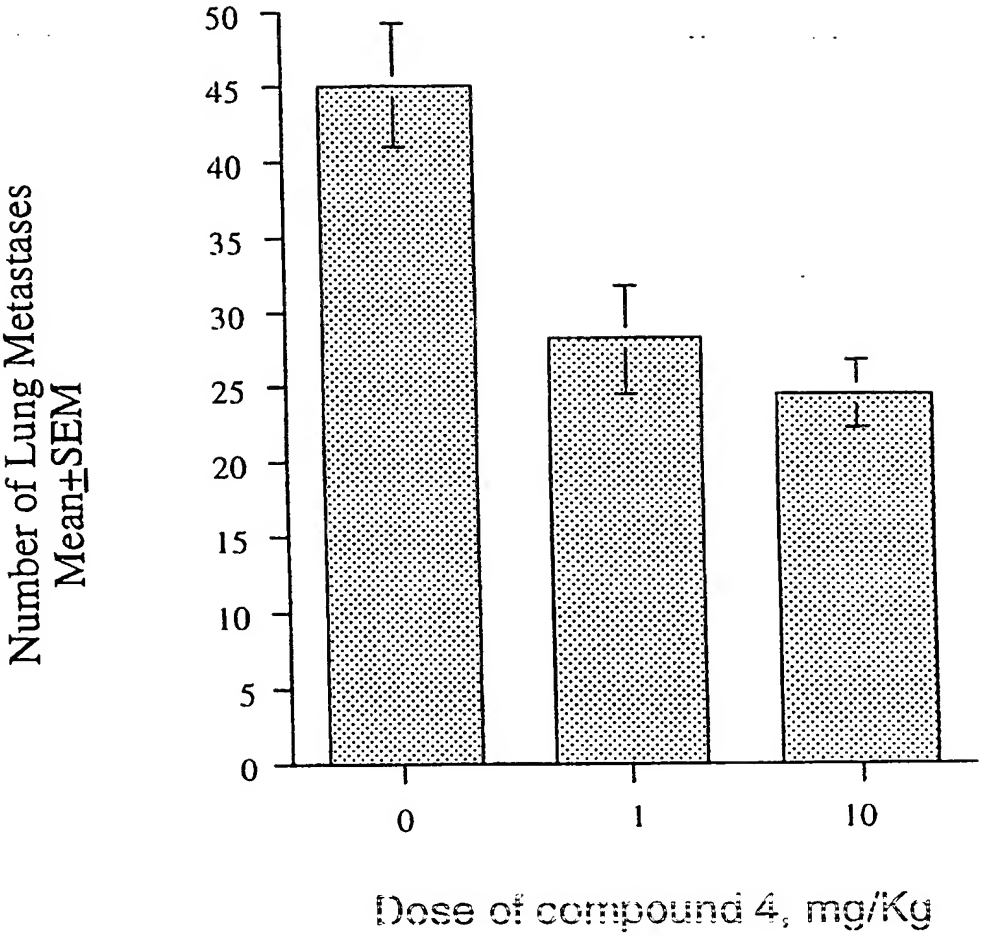


Fig. 4

INTERNATIONAL SEARCH REPORT

Inter anal Application No
PCT/CA 98/01036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D311/72 A61K31/335

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 113, no. 23, 1990 Columbus, Ohio, US; abstract no. 212393g, page 808; XP002092804 see abstract & JP 02 149577 A (EISAI) 8 June 1990 ---	1,2,4
X	JIRO TAKATA ET AL.: "PRODRUGS OF VITAMIN E.1." JOURNAL OF PHARMACEUTICAL SCIENCES., vol. 84, no. 1, January 1995, pages 96-100, XP002092803 WASHINGTON US see page 96 - page 99 --- -/--	1,2,4

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 1999	23/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Francois, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte: ional Application No
PCT/CA 98/01036

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 71, no. 3, 1969 Columbus, Ohio, US; abstract no. 11834s, JANICKI,J.: "ANTIOXIDANT PROPERTIES OF THE A-TOCOPHEROL ESTERS OF AMINO ACIDS." page 206; XP002092805 see abstract & PRZEM. SPZYW., vol. 22, no. 1, 1968, pages 25-26, POLAND	1,2
P,X	CHEMICAL ABSTRACTS, vol. 129, no. 26, 1998 Columbus, Ohio, US; abstract no. 343611e, ARYA,P. ET AL: "DESIGN A. SYNTHESI OF ANALOGS OF VITAMIN E." page 508; XP002092806 see abstract & BIOORG. MED. CHEM., vol. 8, no. 18, 1998, pages 2433-2438,	1-7

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 98/01036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: 8
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 8 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.